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Short Communication

A possible water-soluble inducer for synthesis of cellulase in *Aspergillus niger*

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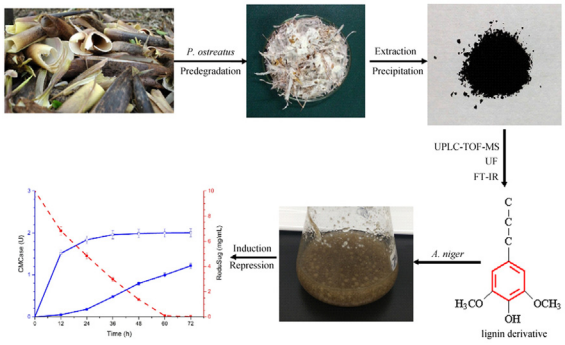
HIGHLIGHTS

- Pretreatment with *P. ostreatus* could promote the production of cellulases.
- Bamboo-shoot shell could be converted into soluble inducer of cellulases.
- The inducer maybe a lignin derivative without aromatic ring.
- The revelation is useful for cellulase industry.

GRAPHICAL ABSTRACT



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ABSTRACT

The synthesis of cellulase in filamentous fungi can be triggered by several inducers. In this study, a bamboo-shoot shell pretreated with *Pleurotus ostreatus* could promote the formation of cellulases in *Aspergillus niger*. Further identification, including UPLC-TOF-MS, ultrafiltration, and FT-IR, denoted that the soluble inducer was not a traditional disaccharide but a type of modified lignin polymer. This revelation may result in incipient strategies to ameliorate cellulase productivity.

1. Introduction

Cellulases are important enzymes and can be applied in several industrial processes, such as food processing (Ma and Mu, 2016b), lignocellulose hydrolysis (Lee et al., 2011) and the synthesis of pharmaceuticals (Wang et al., 2016). Fungi are the major source of cellulolytic enzymes (Lee et al., 2011). For example, *Aspergillus niger* is an efficient producer of cellulases, and it has been exploited in industry to produce cellulases (Hanif et al., 2004). High-yield cellulase production requires a corresponding substance as an inducer. At present, induction mainly depends on insoluble substrates resulting from lignocellulose degradation (Himmel and Bayer, 2009). Considering the ease of manipulation and the complication of separating enzymes from insoluble substrates, soluble

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inducers are usually preferred or required (Xu et al., 2014). It has been reported that D-xylose, cellobiose, gentiobiose, lactose and sophorose (Tani et al., 2014) could induce cellulase formation in some cellulolytic fungi. Nevertheless, adding pure inducers is not a preferred option in commercial applications due to their high expense.

Bamboo-shoot shell is a type of by-product of the agriculture and forestry processing industry (Ye et al., 2014). Cellulose, hemicellulose and lignin account for the majority. It was estimated that the production of moist bamboo-shoot shell exceeds millions of tons every year in China (Qing et al., 2016), but most is discarded as agronomic waste. Compared to other hard or soft woods, moist bamboo-shoot shell contains not only lignocellulose but also water-soluble carbohydrates, pectin and amino acids (Jin and Yuan, 2012). Because of its rich nutritional components, bamboo-shoot shell can be used as a complete medium to culture microorganisms such as *A. niger* (Jia et al., 2011). Furthermore, the derivatives from components of bamboo-shoot shell can be used as feedstocks for other conversions (Qing et al., 2016; Ye et al., 2015; Zheng et al., 2016).

In this study, the effect of pretreatment with *Pleurotus ostreatus* on the production of CMCase was investigated. This pretreatment could promote the formation of cellulases in *A. niger*, and the "true inducer" molecules were derived from lignin polymers. A bamboo-shoot shell could be developed into a water-soluble inducer for the production of cellulases.

2. Materials and methods

2.1. Organism and inoculum

Both *Pleurotus ostreatus* (CCTCC AF 92003) and *Aspergillus niger* (CCTCC AF 91005) were purchased from the China Center for Type Culture Collection and initially cultured on potato dextrose agar (PDA) slants. A mycelia suspension of *P. ostreatus* was obtained by grinding a mycelia mat in an appropriate amount of sterile saline. A conidial suspension of *A. niger* was prepared by adding sterile saline to the slant, and the concentration of conidia was adjusted to 10^6/mL.

2.2. Preparation and pretreatment of bamboo-shoot shell

Moist bamboo-shoot shells were thoroughly washed in running tap water and dried in a hot-air oven at 60 ± 5 °C to a constant weight. The dried shells were milled, and the chips were passed through a 20-mesh sieve. The powder was called NBSS (Native Bamboo-shoot Shell).

The preparation of the nutritional solution followed the description by Vane (2003). Eight grams of NBSS were humidified with 13.0 mL of nutritional solution and autoclaved at 121 °C for 1 h. The sterilized substrate was inoculated with 1 mL of mycelia suspension of *P. ostreatus* and incubated at 28 °C for 15 days. The culture was oven-dried at 80 °C until a constant weight was reached. The dried culture was ground, and the powder was named PBSS (Pretreated Bamboo-shoot Shell).

2.3. Extraction of PBSS

One gram of PBSS was boiled for 20 min with 100 mL water. The mixed solution underwent gauze filtration and centrifugation (20 min, 12000*g*). The supernatant was named Es-PBSS and preserved for the next experiments. Es-PBSS was mixed with 2× volume of absolute ethanol and stored at 4 °C overnight followed by a centrifugation step (10 min, 12000*g*). The precipitate was air-dried at 60 °C and named EPBSS.

2.4. Growth experiments

The basal medium was composed of 5.0 g/L NaCl, 2.0 g/L $(NH_4)_2SO_4$, 1.0 g/L KH_2PO_4, and 0.5 g/L $MgSO_4{\cdot}7H_2O$. Carbon sources were added individually to batches of basal medium to reach a concentration of 10.0 g/L. Additionally, when 100 mL of Es-PBSS was added as a carbon source, the equal water was removed. All media were adjusted to pH 6.5 with 1 M NaOH or 1 M HCl and were dispensed in 100 mL aliquots into 250 mL Erlenmeyer flasks in triplicate. The media were autoclaved at 121 °C for 30 min and inoculated with 1 mL of a conidial suspension of *A. niger* and incubated at 30 °C for 72 h. The sample flasks were withdrawn after 12 h intervals and centrifuged (10 min, 12000*g*) to remove the insoluble substrates.

2.5. Induction experiments

Induction experiments were carried out following the description by van Peij et al. (1998). Briefly, *A. niger* conidia were pregrown in 250 mL Erlenmeyer flasks on a rotary shaker (180 r/min) at 30 °C in 50 mL of basal medium (the same as Section 2.4) with sucrose as the carbon source for 48 h. Mycelia were harvested by filtration and washed twice with basal medium. Equal amounts of mycelia were then transferred into 100 mL of basal medium containing 0.05 g inducers and incubated under previously described conditions.

2.6. UPLC-TOF-MS analysis

UPLC-TOF-MS analysis was based on the methods of Su et al. (2012). Es-PBSS was conducted on a Platisil NH_2 column (4.6 × 250 mm, 5 μm) (Dikma Technologies Inc., USA) using a gradient method (0–1 min, 80% CH_3CN/H_2O; 1–5 min, 85%; 5–30 min, 70%) with a flow rate of 1.0 mL/min. The MS parameters included a spray voltage of 2.5 kV with a source temperature of 110 °C, a desolvation nitrogen gas temperature of 350 °C with a flow of 200 L/h, and a cone gas flow of 40 L/h. The ESI^+ ion was monitored with a transition of *m*/*z* 100–1000 and a cone voltage of 100 V.

2.7. Ultrafiltration experiments

The ultrafiltration was conducted according to the modified method described by Kang et al. (2012). Briefly, 1000 mL of Es-PBSS was filtered using a 1-/5-kDa molecular weight cut-off (MWCO) membrane (MSC300; Shanghai Mosu Science Equipment Co. Ltd, China). The permeate and retentate solutions were recovered to the initial volume with distilled water.

2.8. Fourier-transformed infrared spectroscopy (FT-IR) analysis

FT-IR analysis was based on previous methods (Ma and Mu, 2016a). EPBSS were thoroughly mixed with KBr (1:99, w/w) and pressed to form a disk less than 1 mm in thickness. The FTIR spectra were obtained using a Nicolet 67 Fourier-transform infrared spectrometer (FT-IR) (Nicolet Instrument Co., USA).

2.9. CMCase assays

The appropriately diluted culture supernatant was incubated with CMC-Na in 0.05 mol/L citrate buffer (pH 4.8) at 50 °C for 30 min as described previously (Li et al., 2014). One U of CMCase was defined as the amount of enzyme that produced 1 mg of reducing sugar in 1 h per mL of supernatant.